A STUDY OF THE SPORANGIA AND GAMETOPHYTES OF SELAGINELLA APUS AND SELAGINELLA RUPESTRIS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XXXI.

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(WITH PLATES V–IX)

SELAGINELLA APUS.

SPORANGIUM.—It is by no means an easy matter to determine the origin of the sporangium in *S. apus*. It is quite possible, from a number of slides, to select a series which shall seem to prove that the archesporium consists of two superficial cells originating just above the axil of a sporophyll (*figs. 3, 4*). It is equally easy to procure evidence that a single epidermal cell initiates the sporangium (*figs. 1, 2, 5*). This single cell may be either upon the sporophyll or removed by several intervening cells from its base. The exact line of demarcation between sporophyll and axis is indeterminate. The difficulty lies in the fact that until the sporangium is well established, consisting of some half dozen or more cells, there are almost no indications in the structure, or size, or staining qualities of these cells to distinguish them from the vegetative tissue. Moreover, a sporophyll originates in close proximity to a sporangium (*fig. 5*), and at about the same time and until it has established an apical cell there is no way of distinguishing it from a young sporangium. These facts render cross and tangential sections well nigh useless for an interpretation of the earliest stages. From serial radial, *i. e.*, vertical, sections of the strobilus, if cut with due reference to the phyllotaxy, it is possible to form conclusions by comparison of the series of sporangia and sporophylls in the same rank and of different ages that appear in an exact median section. It is evident that the number of such

sections which may be obtained by ordinary means is very limited. I found it necessary to imbed the strobili singly, examine each section as its plane approached the median region, and constantly alter the angle of the paraffin block in the microtome, until by the vascular strand of the axis and the leaf traces of the older sporophylls I could estimate approximately the section sought. Even with these precautions, I was not infrequently doubtful as to whether a cell that appeared in the right place theoretically for an archesporium might not be the initial cell of a sporophyll from the next rank that a slightly oblique section would display. These explanations are necessary, for after much painstaking study of many sections of tips of very young strobili, I find myself becoming less certain of there being a definite rule governing the initial phases of the sporangial growth. In many median longitudinal sections cut in the manner described above, a single epidermal cell projects from the surface in a vertical line between the youngest sporophyll and the apical cell of the strobilus. Occasionally it takes a deeper stain than the neighboring cells. There are usually from ten to twelve cells between it and the apical cell, and two or three between it and the subtending sporophyll (*fig.* 5). Quite as often this projecting papilla is composed of *two* cells of equal size lying in the vertical line (*figs.* 3, 4). In either case periclinal walls are formed, cutting off one or two cap cells (*figs.* 2, 5, 6). From the hypodermal cell or cells, thus formed, originates the sporogenous tissue. From the cap cell, which divides much more rapidly, the sporangium wall is formed. It is possible, I think, to determine in sporangia of various stages, nearly up to maturity, whether in a given case it originated from a single epidermal cell or from the two superimposed cells. In the second case, the complex of cells resulting from each of the two primary cells consists of more regular radial rows, and there is a quite definite plane of cleavage between the two groups. The only other interpretation of appearances like *figs.* 6 and 8 is that the primary single archesporial cell divided first anticlinally, thus producing the superimposed cells, each of which then cuts

off a cap cell. I have sought in vain, however, to establish that the first two are sister cells. When the sporangium consists of from four to six cells (*figs. 6, 7, 8*), the hypodermal cells assume a different appearance when stained, which distinction is maintained thenceforth in the development, and establish their identity as primary sporogenous cells. There is no apparent regularity in the order or plane of their division, with the exception above stated. The tapetal cells are differentiated very early in the history, and are the peripheral sporogenous cells which assume a more symmetrical shape and regular arrangement (*fig. 10*). A few of the cells in this layer, lying directly above the pedicel of the sporangium, appear to be derived from the vegetative cells in that region, and are not the lineal descendants of the archesporial cell.

Simultaneously with the differentiation of the tapetum the sporangium wall divides into two layers by anticlinal partitions (*fig. 10*). The outer layer soon surpasses the inner in the size of its cells (*fig. 11*). A pedicel develops by the multiplication of the sterile cells next to the axis and beneath the sporogenous mass. The megasporangium cannot be distinguished from the microsporangium until the moment when the cells of the sporogenous tissue cease to divide, separate from one another, and float in the sporangium. At this period, if one or two cells are more regular in form, stain more deeply, and possess a larger nucleolus, they are megaspore mother cells (*figs. 13, 35, 36*). The microspore mother cells are not distinguishable until the karyokinetic figures develop that precede their division into spores. A comparative count of the microspore mother cells in this stage and the cells in a corresponding megasporangium indicates that there is at least one more period of cell division in the former than in the latter. A cytological study may determine other means of identifying the two.

MEGASPORES.—The megaspore mother cell becomes nearly isodiametric, enlarges, stains deeply, and lies near the tapetum (*figs. 13, 35*). Apparently the mother cell never originates far within the sporogenous tissue but always near its periphery.

Infrequently two mother cells occur, and these may go through all the later stages of development, thus forming eight spores in a megasporangium. Normally the membrane of the single mother cell becomes distended by the imbibition of fluid that has been poured into the sporangial cavity from the tapetum. Two successive divisions follow each other with great rapidity. The spindle of the first division I have seen but a few times; the two that follow, I saw more frequently (*figs. 16, 17*). These are extremely small and delicate, occupy the middle of the cell, and are soon much obscured by fibers which arise in the surrounding protoplasm. These fibers at first radiate in all directions from no definite center, but later assume the form of a sextuple spindle with the four daughter nuclei at the poles. No cytological work was attempted, beyond determining the method by which the spores originate, but it is evident even from a cursory examination that there is some connection between the enveloping spindle fibers and the two lumps lying against the nucleus which are represented in *figs. 11, 13, 15*. In the meantime there has appeared a new membrane just within the mother cell wall (*fig. 15*). It is very delicate, but of unequal thickness, and can be detected only in particularly fortunate sections. Whether this membrane arises by cleavage from the mother cell wall, or *de novo* from the protoplasm of the cell, is necessarily a matter of speculation in an object so small. Nuclear plates form across the spindles, and the mother cell divides into four spores tetrahedrally arranged. At the moment of their separation the nuclei lie near the bases of the spores, which correspond in position to the poles of the sextuple spindle, but soon move toward the apices (*figs. 16–18, 22*). As the spores increase in volume, by their pressure outward, and by a folding inward on the part of the irregularly thickened special mother cell membrane above described, the tetrad presents the appearance of a four-lobed body surrounded by a single envelope whose continuity is not broken, floating in the fluid that fills the distended spore mother cell membrane (*figs. 17–19*). Either the special mother cell membrane itself thickens, or a new coat is

formed upon its inner surface. This is the exospore (*figs. 20–22*). The spores increase in volume, but the exospore expands so much more rapidly than does the protoplasmic content, that the latter is left in the apical portion of the spore cavity as a tenuous spherical vesicle of protoplasm filled with a limpid fluid (*fig. 37*). A single very small nucleus, in which with difficulty may be seen one or two nucleoli, lies in the region nearest the spore apex. The rest of the spore cavity between the vesicle and the exospore is filled with a limpid fluid of the same nature as that which occupies the space between the tetrad and the mother cell membrane. Soon hair-like radiations appear traversing these fluid regions (*figs. 42, 43*), whose general direction is from the spore mother cell membrane toward the center of the tetrad. A thick layer which stains very deeply appears upon the inner face of the exospore (*fig. 37*). The latter maintains its more rapid rate of growth and soon afterward is widely separated from this layer by a space filled with the liquid and the radial fibrillae (*figs. 38, 42*). The sculpturing of the exospore begins almost immediately upon its inception. The spines are laid down by depositions of matter derived from the liquid between the exospore and the mother cell membrane (*figs. 42–46*).

As the megaspores increase in size, they are forced apart by the liquid in which they float seeping in between them (*figs. 38, 42, 43*) and the exospore is ruptured between the spores in such manner that each presents the appearance of a tetrahedron with a hemispherical base and three plane triangular faces. The tearing apart of the spores leaves a trefoil-shaped cleft extending from the apex along the three ridges between the triangular faces, and bounded by the flaring flaps of the torn exospore (*fig. 58*).

The sterile mother cells in part disappear by dissolving in the slimy fluid in the sporangium cavity, but not until the exospore is well developed. Some persist however throughout the development of the gametophyte and perhaps may grow slightly; they never divide.

FEMALE GAMETOPHYTE.—The spore is but a small fraction of

its final volume when the sexual generation begins, thus overlapping the asexual (*fig. 44*). The initial steps of the female gametophyte development are the rapid expansion, without corresponding increase in thickness, of the protoplasmic vesicle, and the division of its nucleus. The nuclei divide by karyokinesis, and with each successive division become larger (*figs. 45, 46*). The thick envelope surrounding the vesicle stretches, becoming proportionately thin as its surface increases, until it comes to lie against the inner surface of the exospore. At this stage it consists of two distinct layers, the endospore and mesospore, both mere films which may be readily detected, as they stain differently and are easily torn apart (*fig. 46*). The gametophyte at this stage thus consists of (1) exospore, still growing, (2) mesospore; (3) endospore; (4) the protoplasmic vesicle, consisting of a very thin and homogeneous layer of protoplasm applied to the inner surface of the endospore, in whose apical region are imbedded numerous large, ovate, flattened nuclei; and (5) a large central vacuole filled with a watery fluid in which are suspended many oil drops.

Beyond the increase in the number of nuclei, which preserve about the same relative distance from one another, there is no further change until the spore membranes complete their growth. When the maximum size is reached, fibrillae arise in the protoplasmic vesicle at the apex, and radiate downward over its surface, blocking it off into irregular areas, each of which contains one or more nuclei (*figs. 49, 51, 52*). Simultaneously the protoplasm becomes invaded with masses of granular matter, and encroaches on the space occupied by the vacuole. Nuclear divisions take place radially, and so rapid is this process that frequently the spindle fibers of three and four generations of nuclei may be seen in a single section. The fibrillar radiations permeate the protoplasm, keeping pace with its increase in thickness. As the central vacuole diminishes in size, its contents change in appearance from an emulsion to a turbid fluid thickened with granules (*fig. 53*). The protoplasmic fibrillae apparently are concerned with the distribution

of this nutriment before cell walls are formed. They have the appearance of streams bearing granules. The final and penultimate divisions of the nuclei are distinguished by the appearance of nuclear plates and spindles of quite different appearance, which result in the formation of definite cells with cell walls, each containing a single nucleus (*figs. 26–28*). At this stage, each nucleus is so surrounded by a mass of proteid as to be completely obscured. The only cells not thus gorged are a limited number which lie in the upper layer nearest the apex, beneath the trefoil-shaped cleft in the exospore (*fig. 54*). By continual multiplication they form a prothallial cushion, which widens the breach and bulges through. These cells are much smaller than those filled with the food supply. There may be from three to five layers in this cushion. There is no diaphragm between the region designated the prothallial cushion and the mass of storage cells underlying it.

ARCHEGONIA.—A few cells in the prothallial cushion soon become conspicuous by reason of their large nuclei. Each divides by two periclinal walls, forming a tier of three cells. The uppermost of these divides by two anticlinal walls at right angles to each other into four cells. These again, by periclinals, form the four cover and the four neck cells. The middle one of the original tier does not divide and becomes directly the single neck canal cell. The lowermost divides into the egg and the ventral canal cell (*figs. 29–32*). A suggestive irregularity sometimes occurs in the last mentioned division. The central cell, that is, the lowermost cell of the original tier, may divide in such fashion that the egg and its sister, the ventral canal cell, may lie side by side in the venter of the archegonium, instead of in the normal fashion of ventral canal cell above the egg (*fig. 32*). The cover cells project very little from the surface of the prothallium. Thus the archegonia are imbedded in the surrounding tissue, whose cells in immediate contact with the egg and the ventral canal cell become more or less modified in form. The neck canal cell pushes up like a wedge, spreads apart the four neck cells, and dissolves. The ventral canal cell

also disappears (with a possible exception noted hereafter), and the egg lies free in the venter. There is a large receptive spot on the oosphere, and its nucleus is not centrally placed (*fig. 33*). I have never seen more than five archegonia in a single gametophyte.

FERTILIZATION.—Not only are the archegonia formed in the unshed spores, but frequently, at least, fertilization and the early phases of the sporophyte development take place while the sporangium with its prothallus are still in the strobilus. The strobili cease to grow, fade, and may separate from the plant before fertilization, but the spores do not fall from the sporangium. It was not until I had collected from the soil several hundred spores which had been shed, with the expectation of finding fertilization stages, that I thought to examine the withered strobili. Almost without exception in these I found embryos, whereas *in no case* have I found any evidence of fertilization in the spores that are shed.

FURTHER DEVELOPMENT OF THE MEGASPORANGIUM.—The glandular tapetum is very active until the megaspores have stored their maximum amount of nutriment for the growth of the embryo. At this stage they quite fill the sporangium cavity, which in consequence has assumed a four-lobed appearance. The tapetum then declines in importance. Its cells collapse and form a pavement-like layer. The outer layer of wall cells becomes greatly modified. In section it appears precisely like a similar section of the annulus of a leptosporangiate fern (*fig. 52*). Four areas of the larger thick-walled cells, corresponding to the protuberances caused by the spores lying within, are separated from one another by narrow strips of small cells with thin walls. The latter are the lines of dehiscence. The sporangium splits into two valves along these lines, but the halves do not separate so widely as to allow fertile spores to escape. Apparently they may open and close more than once. The sporangium appears fresh and active, and its wall contains chlorophyll until after fertilization has occurred. With the decline of the tapetum the lower stratum of the wall becomes

more vigorous, as does a group of cells that lies just above the pedicel, and which projects into the sporangial cavity (*fig. 58*). This cushion is in close relation to the vascular strand and probably facilitates the supply of nutriment to the sporangium wall, until the embryos begin to form.

Frequently I have found microspores (in which the spermatozoids had formed) within the megasporangium at the period of fertilization, and it is possible that the microspores are hurled into a gaping megasporangium when ejected from the microsporangium.[1] This is rendered more probable by the frequent occurrence of microspores caught in the angles between sporophylls and stem. Moreover, if plants that have become somewhat dry be profusely watered, the mature microsporangia open explosively and discharge spores.

MICROSPORES.—The microspores of *S. apus* are much smaller than those of *S. Kraussiana* and *S. Martensii*. They early develop a pebbled, thick exospore, which causes much trouble in imbedding and sectioning (*fig. 69*). Moreover, a comparatively small number, in proportion to the immense output, mature. Curious aberrations in growth are constantly found. It was necessary to study mature gametophytes discharging spermatozoids and trace back the different stages to the mother cells. Frequent comparative measurements finally afforded a clew to detecting abnormalities in the early stages.

Not more than five sixths of the potential mother cells divide into spores; the others rapidly disappear. The division is accomplished in a manner analogous to the division of the megaspore mother cell, which is very little larger. Two spore coats develop, a thick spiny exospore, and the delicate membranaceous endospore. The microspores are shaped like the

[1] I have examined *S. Martensii*, *S. Kraussiana*, and *S. denticulata*, growing in the greenhouse, with reference to this point. The two former do not shed their strobili, and I have found loose megaspores containing sporophytes in the soil on the benches where the plants are growing. The last mentioned species, on the other hand, sheds its strobili in profusion. I find, however, that their spores are invariably sterile and aborted, and therefore am unable to form an opinion as to whether this shedding is the normal habit, or due to cultivation in an unnatural environment.

megaspores, but, unlike the latter, separate from one another as soon as the exospore develops. As was the case with the female gametophyte, the sexual generation begins before the microspore has ceased growing. At the moment that the microspores separate from one another, each possesses a thick and a thin coat, a layer of protoplasm parietally placed, with one nucleus, and a central cavity filled with fluid (*fig. 68*). The nucleus increases in size and divides. This process often takes place in that part of the spore where one of the lateral ridges meets the hemispherical base (*fig. 78*). The protoplasm increases in quantity and encroaches on the central vacuole. Granules of various sizes make their appearance in the cavity and in the surrounding protoplasm. These bodies stain precisely like the nuclei and the more regular ones may be mistaken for them, as I frequently discovered in the early period of my own work (*fig. 77*). By precautions in decolorizing, the presence of nucleoli always distinguish the nuclei from the granular masses. These bodies are formed by the agglomeration of many smaller granules. One of the nuclei formed by the first division remains against the wall. It may grow larger and the protoplasm immediately surrounding it is somewhat denser, but no wall separates it from the rest of the spore. This may be the vegetative prothallial cell (*figs. 70, 75*). The other nucleus passes into the center of the spore. The protoplasm that envelops it sends out radiating processes that incompletely divide the spore cavity into irregular chambers, each of which is filled with granular masses of various sizes and shapes (*figs. 76, 80, 81, 83*). These strands of protoplasm are continuous with a thin layer in contact with the endospore. The central nucleus with its envelope of protoplasm divides into two cells which usually separate from each other (*figs. 79–83*). By repeated division of each of these cells a complex results which consists of two uniform masses of sperm mother cells. There is no law of sequence which the cells follow in dividing, although the final product consists of cells very regularly arranged (*figs. 95–97*). The male

gametophyte then is made up of one prothallial cell and a naked mass of sperm cells, which later come to float in the slime produced by the disorganization of the food granules. Sometimes at this stage the large deeply stained prothallial cell may be seen flattened against the endospore (*fig. 97*). There are about 128 sperm cells. The exospore splits along the three ridges from the apex downward, and the endospore, dilated with fluid, protrudes through the gap (*figs. 98, 99*). The sperm cells separate from one another, and a single spermatozoid is organized in each. These are spirally coiled like those of Osmunda—two complete turns and a part of a third—but I can demonstrate no cilia (*figs. 33, 100*). Neither in appearance nor in movement do they resemble any bryophyte spermatozoid with which I am familiar, or have seen figured. The movement of biciliate gametes is characteristically different from that of an ordinary fern. These spermatozoids progress with a screw-like motion. The latest stages of development occur in the strobili after they are shed.

MICROSPORANGIUM.—At maturity the microsporangium wall consists of two layers of cells, of which the outer is divided into regions of thick and thin-walled cells, which enable the sporangium to open lengthwise into two symmetrical valves (*fig. 62*). The microspores are discharged with much force. If plants that have become somewhat dry are watered copiously and covered with a bell jar, the ripe microsporangia burst open and jerk back their valves, which instantly recoil and hurl the microspores. These may be seen, looking like red powder, lodged in the axils of leaves or upon any other part of the plant where they have chanced to fall. Of necessity the waste must be great. If this is the method adopted by the plant to secure fertilization it may account in a measure for the extremely small number of sporophytes that are developed.

S. apus grows most profusely in a neighboring locality, where almost daily observations have been made during the past year. Early in May 1900, when this work was undertaken, the strobili were well advanced. It was observed that if several eggs in one

strobilus are fertilized, the formation of new sporangia is checked. The sporophylls begin to decay, as finally does the axis immediately below the fruiting head. The production of new strobili went on until late in August, at which time all the strobili were shed, whether fertilized or not. In September vigorous vegetative growth took place during the fall rains, and continued until checked by several days of very cold weather in December. In the latter part of the same month, upon removing the snow that covered the plants to a depth of six inches, they were found to be green and ready to grow at any favorable moment. Large sods were lifted, without disturbing the plants, and brought into the warmth of the greenhouse, whereupon they responded promptly to the blander conditions. After a week, upon gently disentangling the individuals in order not to detach the semi-decayed strobili which were partially covered by the soil, I found several young sporophytes which had thrust their cotyledons and roots through the crevices of the megasporangia. From this it seems safe to assume that an embryo may have two periods of growth separated by one of quiescence, quite comparable to those of seed plants.

SELAGINELLA RUPESTRIS.

There could be hardly a more striking contrast in the external appearance of two closely related plants than exists between these two species of Selaginella that are natives of New England. *S. rupestris* is to be found in the most exposed situations, growing on the granite rocks on the mountain sides, wherever there has been enough weathering to insure the deposition of a little soil in the hollows. For six months of the year they endure alternate drought and drenching, and the frequent and rapid changes of temperature which are characteristic of this region, with no more protection than is afforded by their own structural adaptations. The midsummer conditions are even more trying. The plants that were studied for this work grew on bare rocks upon which the sun beats nearly all day from June to September. The fruiting spikes are to be found in profusion at all times of

the year, but, as will develop later, the spores in the main are sterile. Material was fixed first about June 15, 1900, and at intervals of two weeks thereafter until February 1901. Sods were lifted from the rocks, and planted in shallow boxes which were kept in a warm place exposed to the direct sunlight. The spores were shed freely all summer. I collected other plants from a similar xerophytic situation at Starved rock, Illinois, in August, and for comparison material was sent me in Northampton, Mass., from Austin, Texas, in November. These three regions are 800, 900, and 1600 miles apart. The plants collected in Massachusetts and Illinois, from June until February, almost without exception produced megasporangia only. The Texas material collected in November, on the other hand, was almost purely microsporangiate.[2] Expecting to find prothallia and young sporophytes developing in the spores that had been shed, these were picked out from the loam from which the plants were growing and killed at frequent intervals during the summer, with the purpose of getting all important stages. In all cases these proved to be barren. Late in August—as was the case with *S. apus*—the young sporophytes were discovered protruding from a withered strobilus that was nearly covered by the dirt. Further search produced many well-developed embryos, but it was apparently too late in the season to secure early stages (*fig. 126*).

During the first week in January, a sudden thaw was succeeded by several days of rain. Plants were collected from the mountain and gradually brought into a greenhouse temperature. The old strobili became greener at their tips, and new vegetative shoots started from the lower part of the stems. It is doubtful whether the increase in the number of individual plants in a given locality is due to any considerable extent to sexual reproduction. A hollow in the rock, or a crevice, becomes filled with a closely compacted colony that is the result in great measure of vegetative reproduction, where prostrate branches, under

[2] Some of the Texas material was submitted to Professor L. M. Underwood, of Columbia University, for identification. He informs me that it is not *S. rupestris*, but a closely allied unnamed species.

favorable conditions of moisture, have rooted, and later have severed their connection with the parent plant. In examining many colonies, very few young plants that originate from spores are in evidence, although the soil may be thickly beset with the spores that have been shed. Bits of the old plants that have been torn off by the action of the wind or rain are frequently caught in crevices along the precipices, and it is from these that new clumps are most frequently started.

In *fig. 124* I have endeavored to convey an idea of the structural adaptation of the strobilus to its austere environment. The closely overlapping sporophylls form four rows, in whose axils the sporangia have little space to develop. The growing apex is protected by at least twelve and frequently sixteen sporophylls which envelop it. The epidermis is two or three layers of cells thick on both surfaces of the sporophyll, except in a shallow groove running lengthwise along the middle of the ventral surface. In this groove are the comparatively large crowded stomata, which are protected by the next older overlapping sporophyll of the same row. While the leaves are yet very small the apex ceases to grow and becomes transformed into a branched spine. The formation of the horny epidermis proceeds from the apex toward the base. Between the ligule and the sporangium the tissue retains its meristematic nature. The vascular bundle occupies the central shaft of the strobilus, and is augmented from the apex downward by the leaf traces that join it from the sporophylls. Four large communicating air spaces, spanned by trabeculae, surround the entire system. These communicate with the exterior by certain larger chambers into which the stomata open. There is little closely compacted tissue in either sporophyll or stem. All the cells except the epidermis and the vessels contain chlorophyll. This structure is in marked contrast to the delicate unprotected sporophylls of *S. apus*.

A single superficial cell, which uniformly is so close to the base of the subtending sporophyll that it is impossible to determine whether it belongs to the stem or the leaf, is the origin of

the sporangium (*fig. 101*). The sporophyll immediately above it starts simultaneously from an exactly similar cell. A wall separates the archesporial cell into an epidermal cap cell and a hypodermal cell (*fig. 102*). The sporangium wall develops from the former, the sporogenous tissue from the latter. A few sporogenous cells near the base originate from the stem tissue (*fig. 105*).

The tapetum and sporangium development are quite like those of *S. apus*. The sporophylls and stem apex, on the other hand, have no apical cell. The ligule appears later in the history of the sporophyll than in *S. apus*, and the megaspore mother cell is somewhat larger. Certain cytological features serve to distinguish these cells from the sterile sporogenous cells (*fig. 106*). The division into spores presents some curious variations. Sometimes the spore mother cell divides once only, forming two megaspores; again, after the first division, one of the daughter nuclei may divide, or occasionally both. In any case, so far as observed, but one or two normal megaspores are ever formed; if there are others, they are dwarfed in size and never grow. That the exospore is a membrane common to both spores is evident from *figs. 119, 120, 121*. The formation of the mesospore and endospore is not preceded by any such deeply staining layer as is represented in *fig. 42*. The protoplasmic vesicle expands and overtakes the spore wall. This stage terminates the development of the megaspore (*fig. 123*). In the older sporangia, nearer the base of the strobilus in the specimens that I examined, no further development had occurred beyond increase in size and thickness of the spore coats — the contents showed that the spores were abnormal.

This sequence of events, although followed step by step repeatedly, had been rejected as probably not normal, until the sporophytes were discovered. As the number and shape of the megaspores in these sporangia which contained embryos agree with the stages described, in spite of the gap that remains to be bridged between the megaspore and mature gametophyte, it seems reasonable that the observations can be relied upon as far as

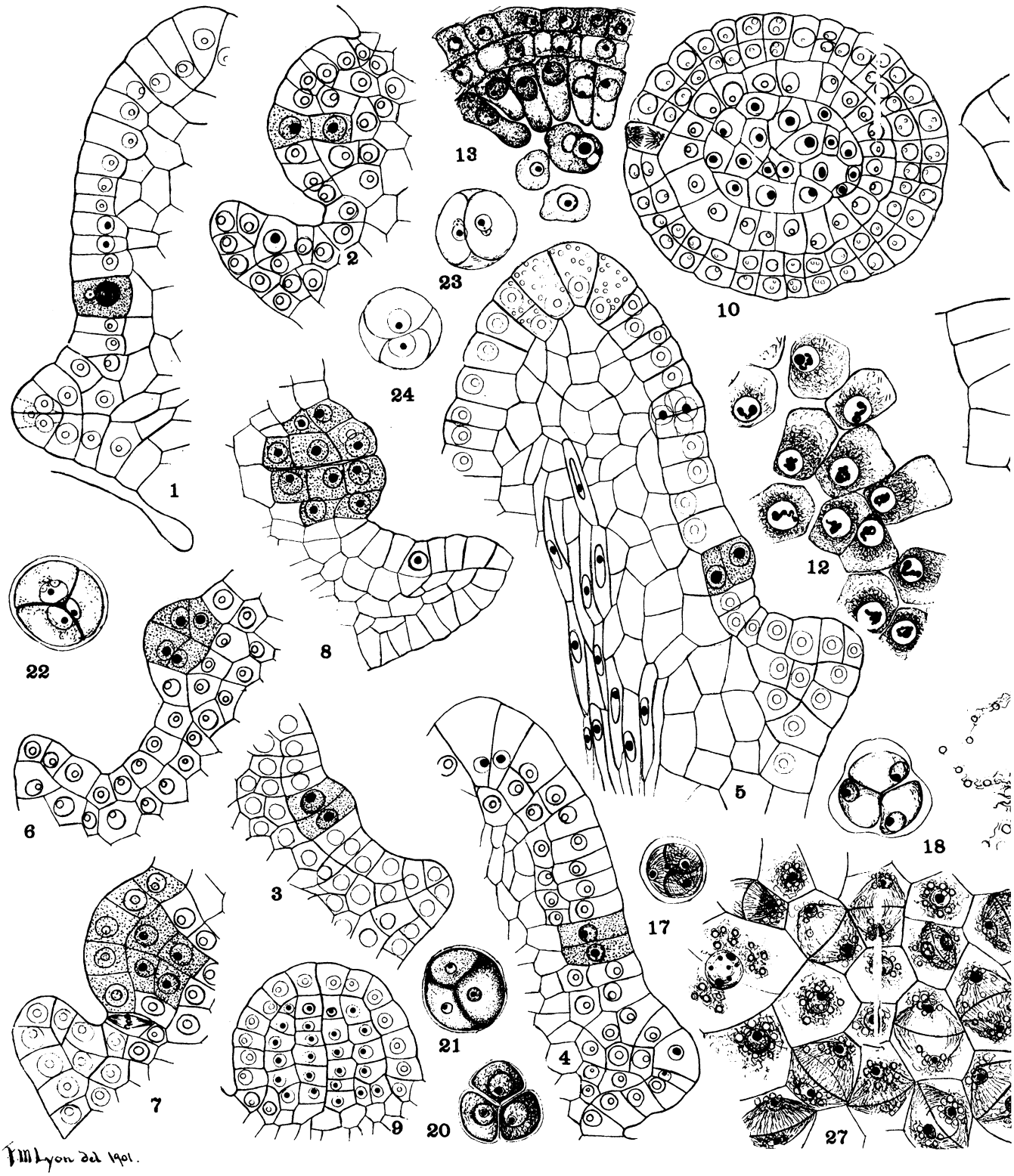
they go (*figs. 108–120*). A very close series of stages from start to finish is necessary to produce confidence in any interpretation of phases of a plant which displays so many irregularities, and evidently has so nearly lost its power of sexual reproduction.

The development of the microspores has not yet been followed in detail. Observations on this subject, together with the growth of the female gametophyte and the embryology, will be given in a future paper. One interesting feature that may be noted now is the incomplete septum which projects into the cavity of the microsporangium (*fig. 125*). This is formed in part from sterilized sporogenous tissue and in part from the cells of the pedicel.

Since the above was written, a package of fresh material has been sent me from Texas. All the plants have begun their spring growth and the winter strobili are growing at the apex. The first sporophylls of the season subtend megasporangia. I find not more than eight or ten in each strobilus. Above these are microsporangia to the number of twenty or thirty. In this material it has been my good fortune to observe the method by which the spermatozoids obtain their entrance to the megasporangia. A perfectly fresh, vigorous strobilus was cut from the plant and stripped of its sporophylls, thus exposing the sporangia *in situ*. A megasporangium, which was observed gaping open and in close proximity to a microsporangium that had discharged its microspores, was separated from the strobilus and examined under the microscope. Six microspores were caught on the sculptured surface of the megasporangium near the edge of the valves, which were slightly separated from each other. One microspore, evidently mature from the fact that the exospore was split and the endospore protruding, suddenly discharged a current of slime which at first proceeded directly away from the megasporangium. Soon, however, as if acted upon by some attractive influence from the gametophytes within, the stream turned abruptly and entered the opening between the megasporangium valves. The spermatozoids were swimming with characteristic rotary motion in this stream. With difficulty, it was

possible to make out that they were similar in shape to those of *S. apus*, but much smaller. To each was attached a vesicle. An attempt to stain them with osmic acid on the slide failed to demonstrate any more details.

ADDENDUM.— Since the foregoing was forwarded for publication it has been possible to verify the main facts and to explain certain apparently contradictory features with regard to *S. rupestris*. The spring and early summer of 1901 were extremely wet as compared with those seasons in 1900. *S. apus* growing in low pastures made few strobili and aborted spores in contrast to the profusion of the year before, whereas *S. rupestris* throve in the unusual supply of moisture and developed strobili, which in turn produced embryos prodigally. The impotency of the megaspores gathered in midsummer of 1900 was obviously due to the lack of water; for at the expiration of the rainy season this year, the last of June, and before the plants had suffered from drouth, they were lifted with all the underlying humus and placed in an open situation in the Botanic Garden. Profuse watering was continued and thorough draining secured. A large percentage of the strobili ripened after fertilization had occurred, and instead of few embryos found with much effort as in the season of 1900, hundreds of young sporophytes have been secured. The sequence of events in a propitious season, based upon the observation of two years, seems in brief to be this: Strobili are formed on the new vegetative shoots of the plant in late summer and autumn. Only megasporangia develop that season, and in these the gametophytes reached the stage bearing archegonia. In the spring, these strobili resume their apical growth, and first microsporangia appear. Thus each strobilus has a basal zone of megasporangia approximately six months old, and above it a narrow region of microsporangia. The number of microsporangia appears to be strictly limited. I have found eight to twelve as a rule. Thenceforth so long as the strobilus continues to grow during the remainder of that season megasporangia only are developed. The production of these is checked in case embryos form in the lowermost zone of



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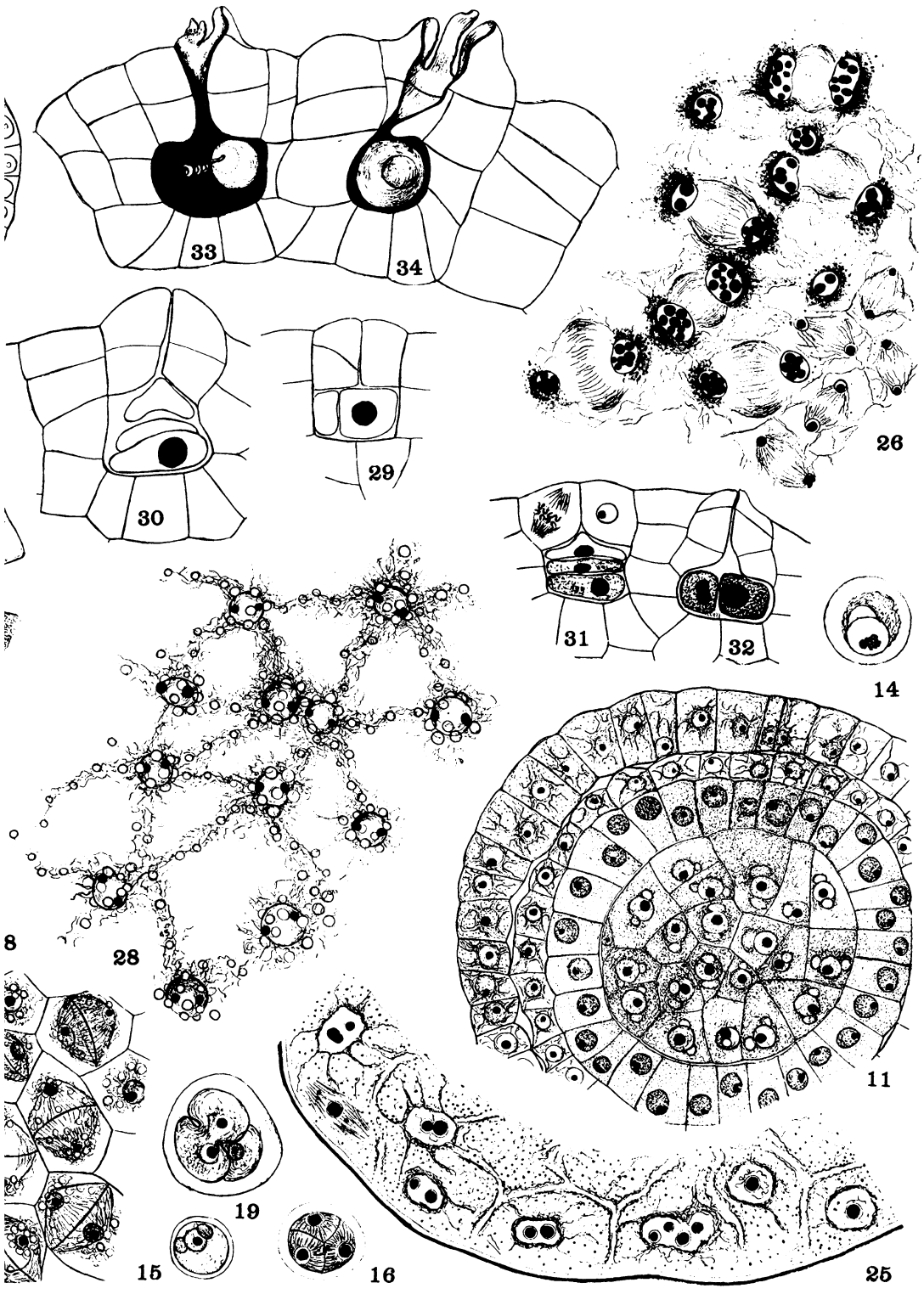

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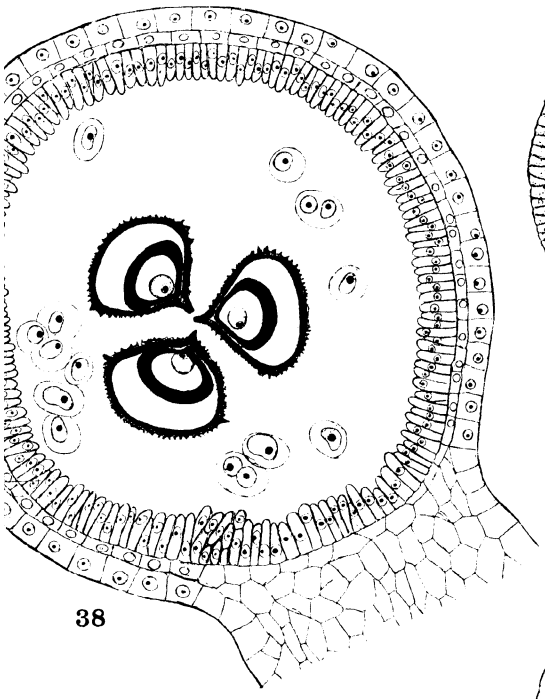

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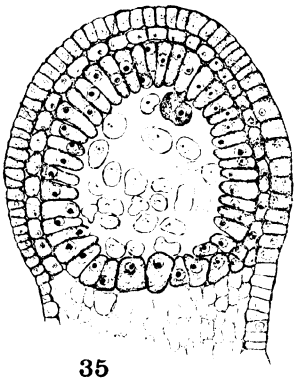

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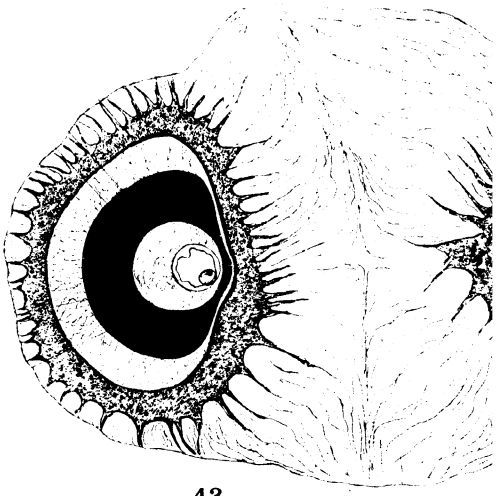

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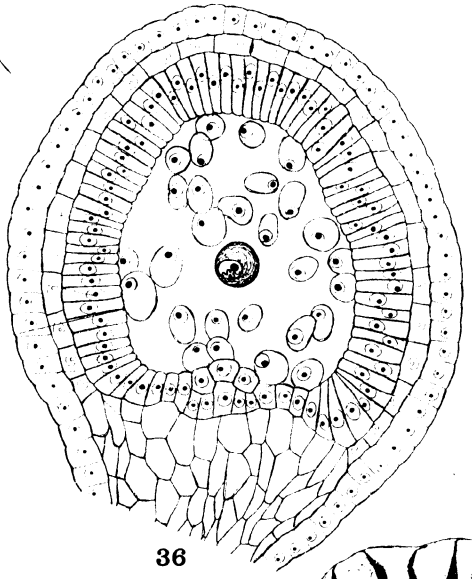

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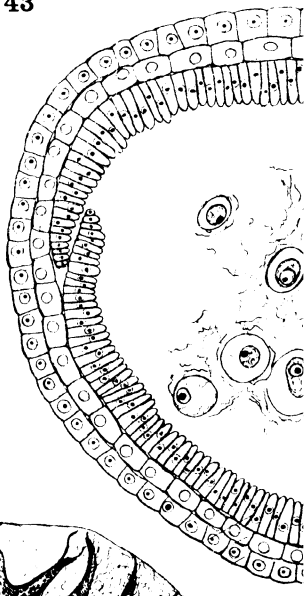

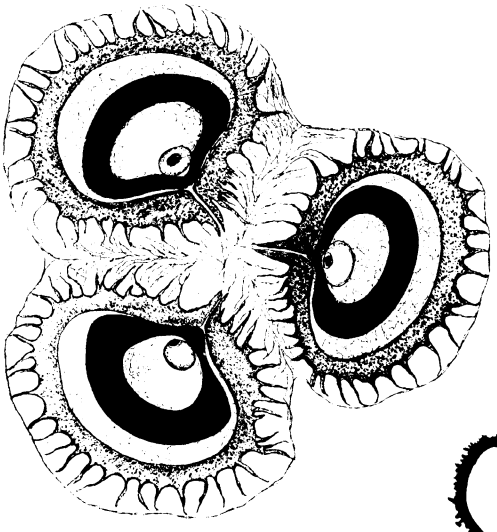

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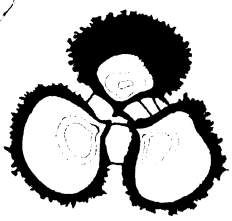

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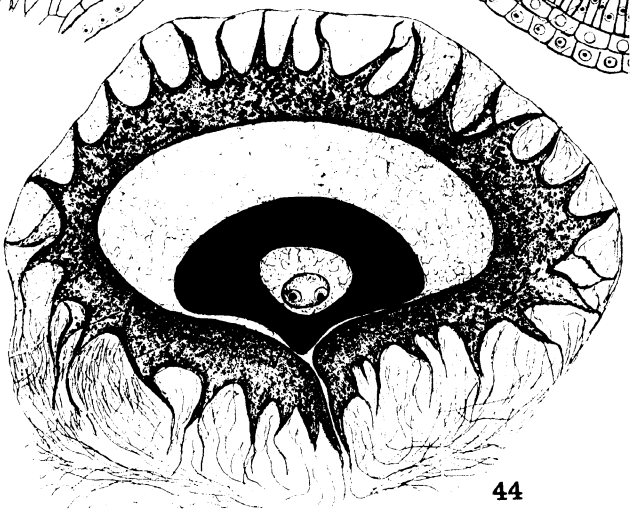

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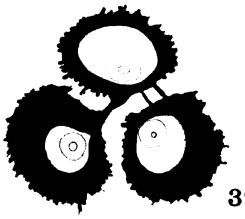

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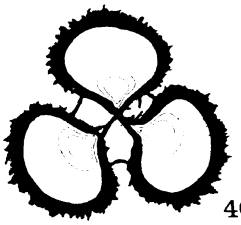

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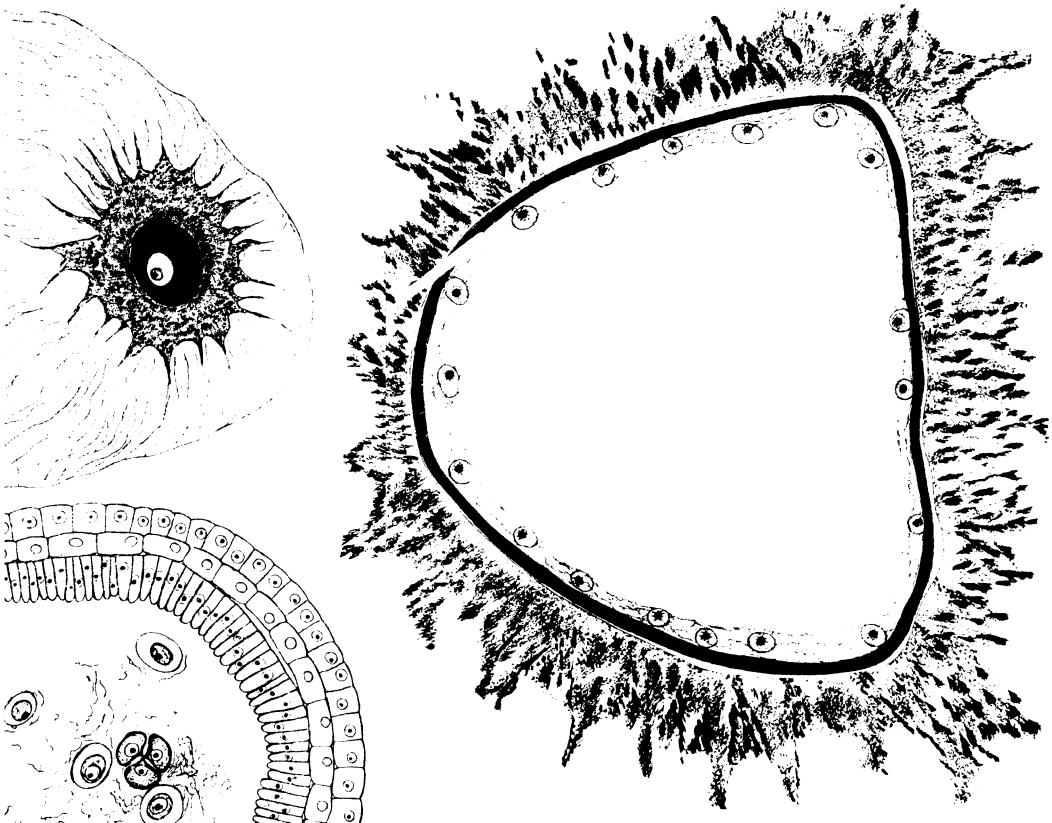

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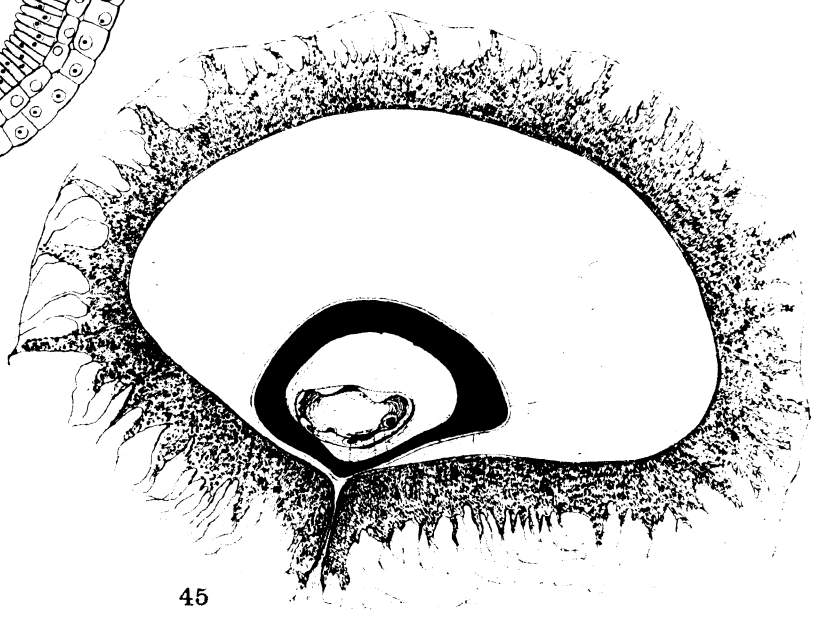

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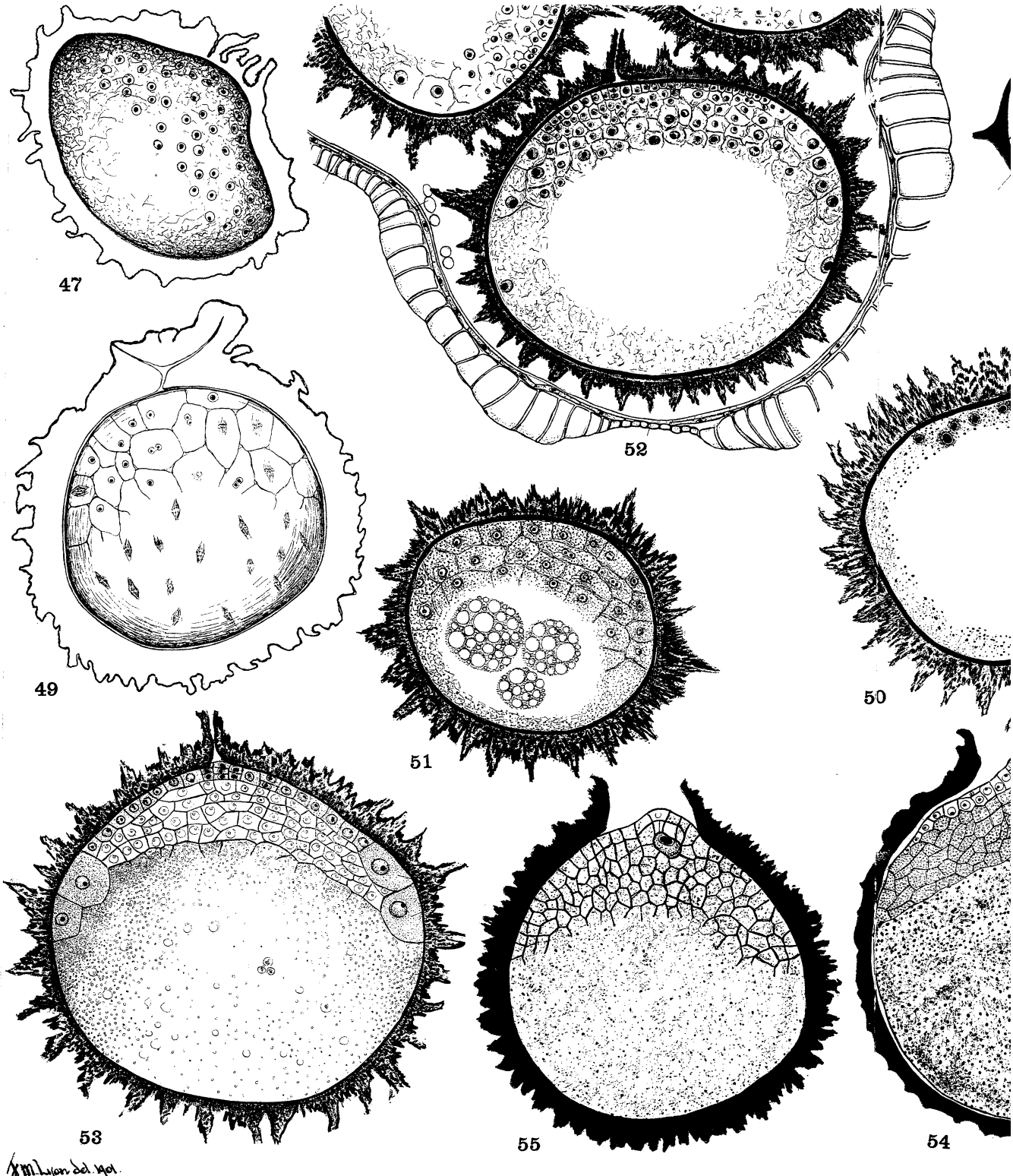

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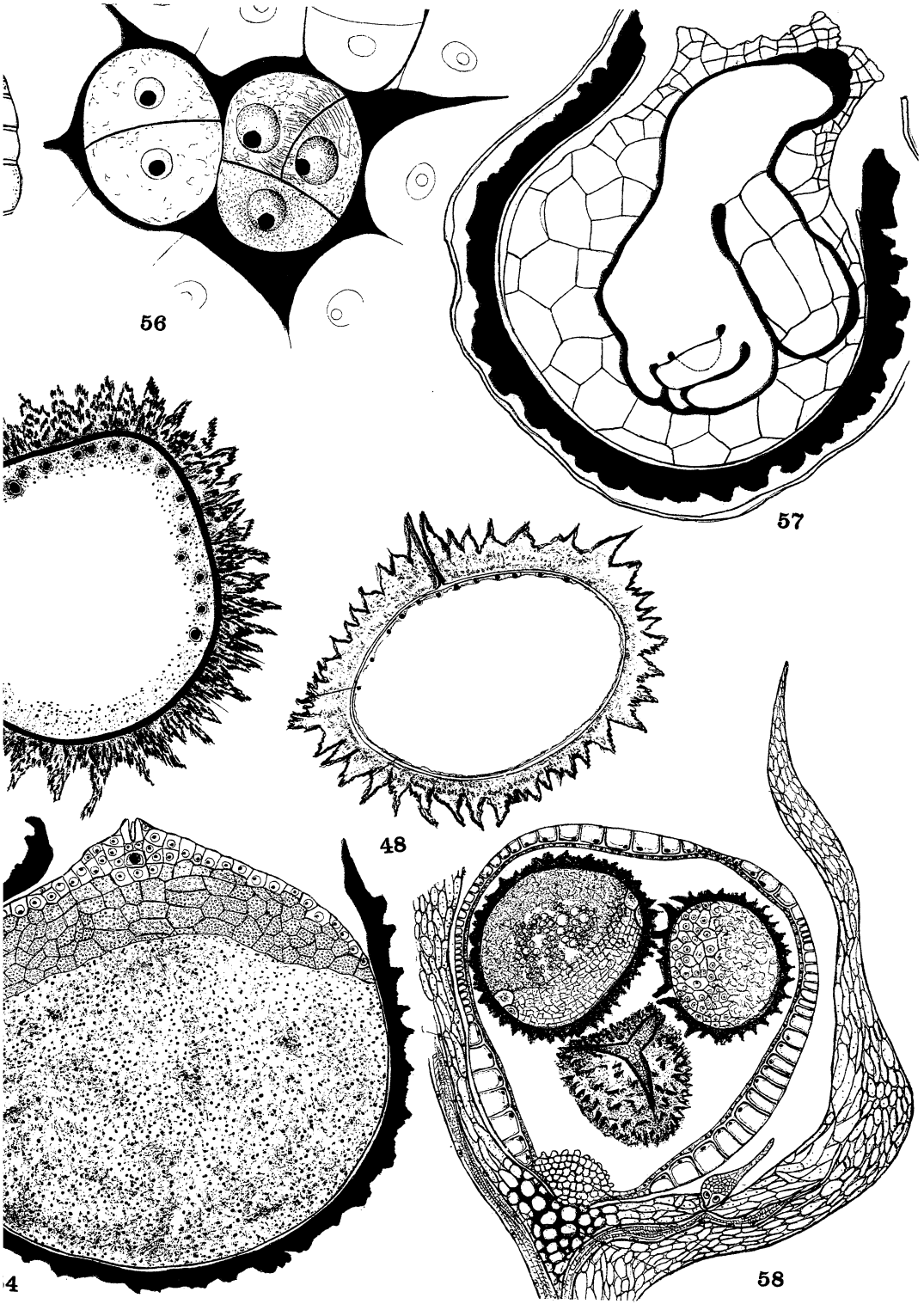

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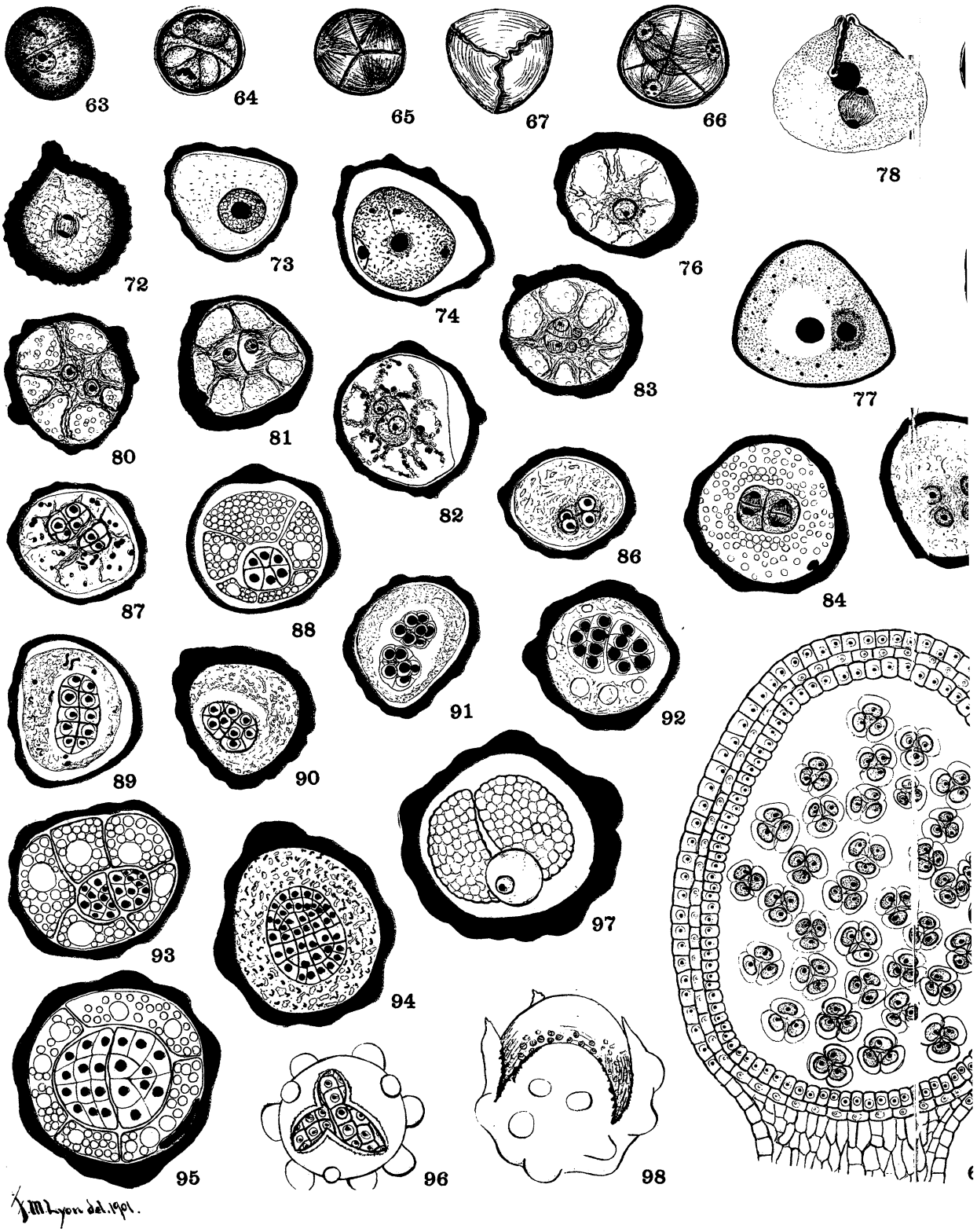

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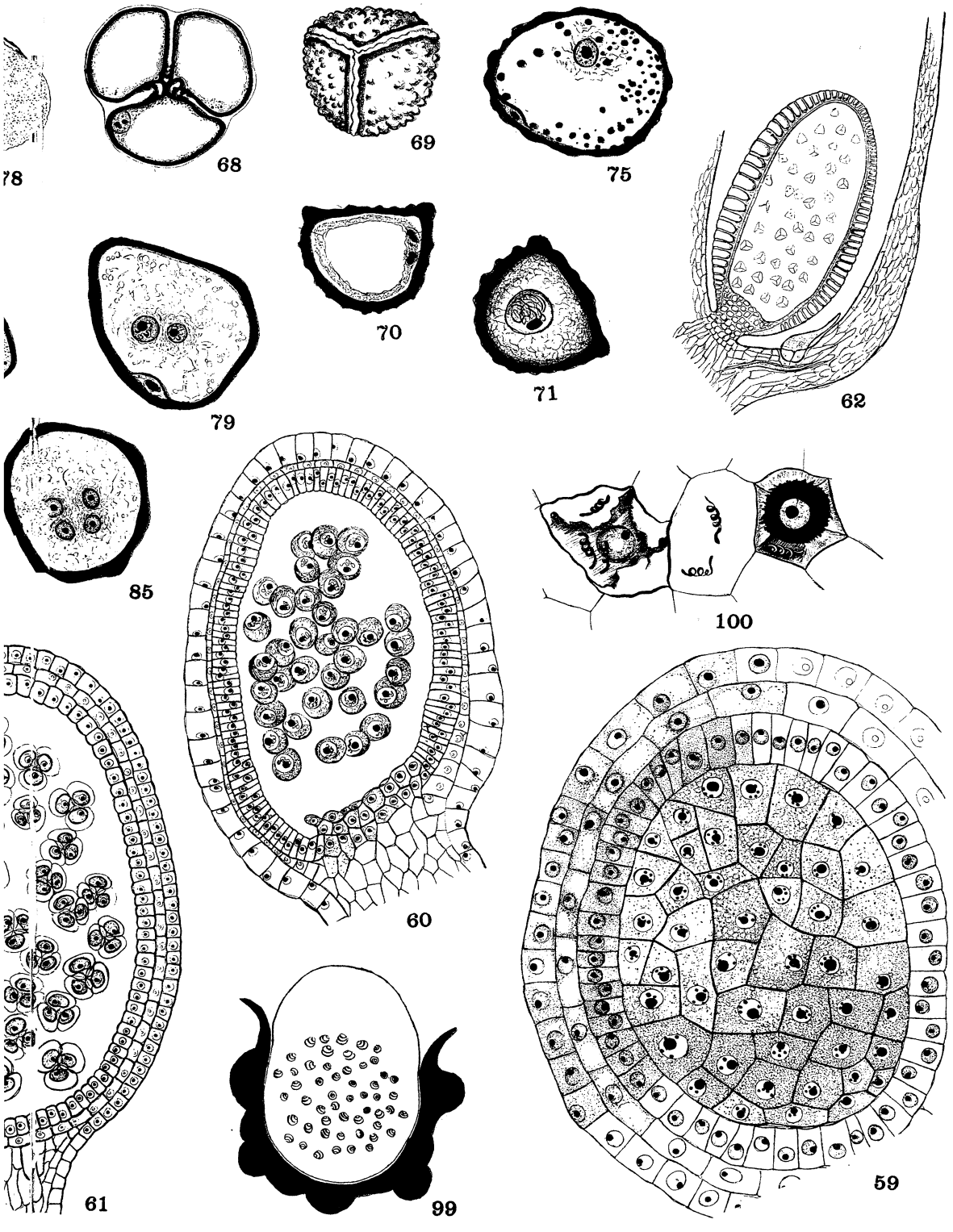

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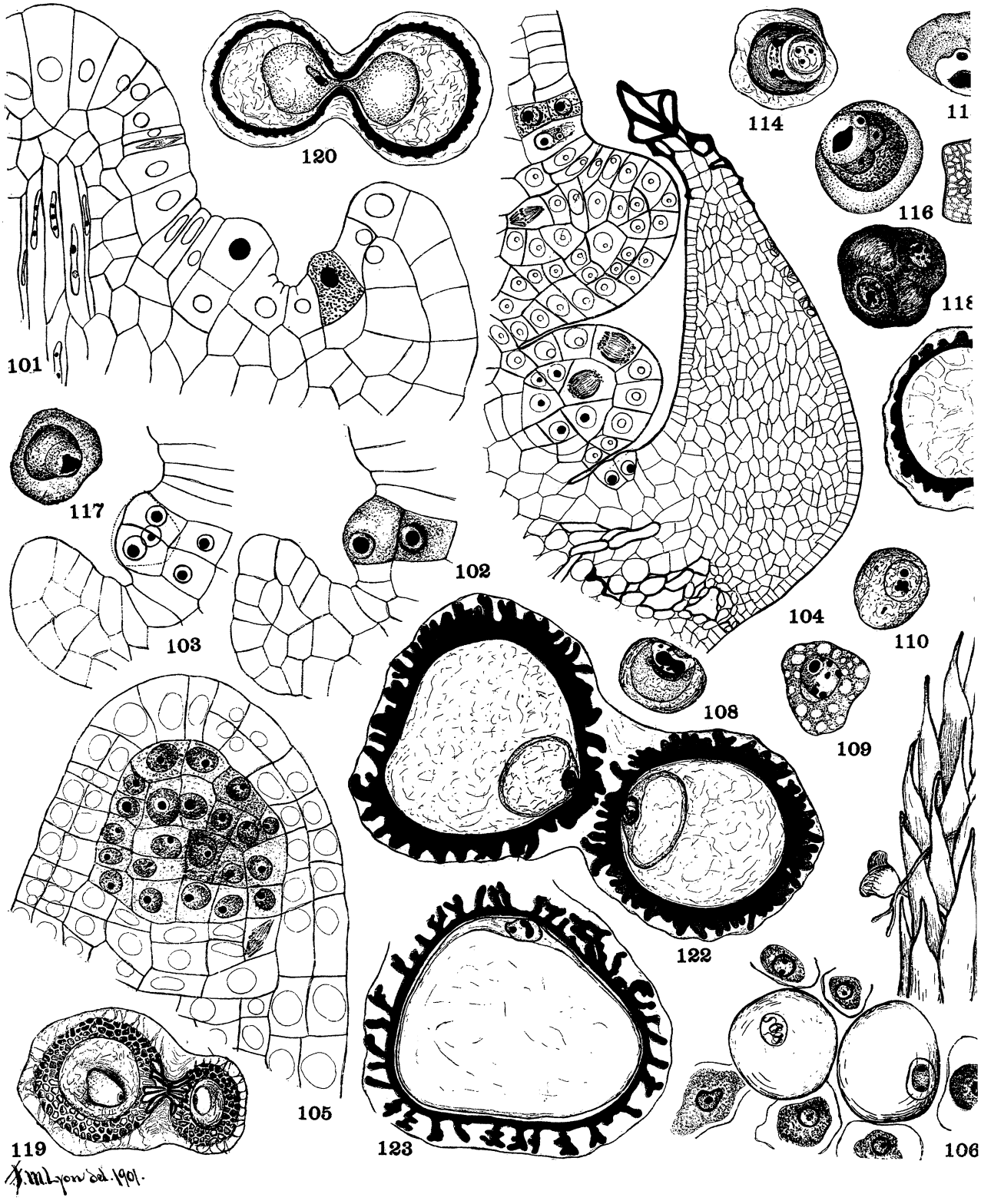

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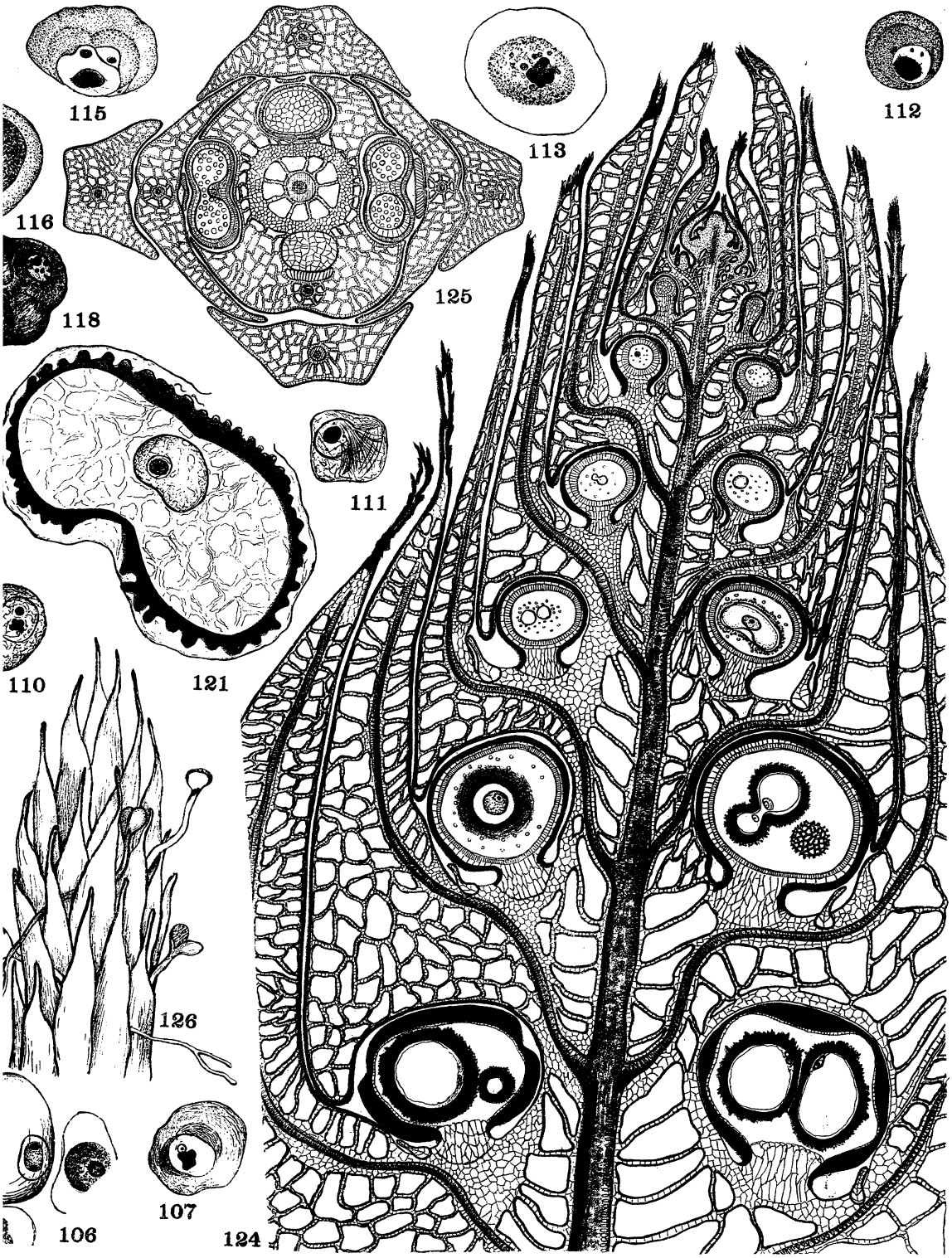

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megasporangia. Immediately upon the cessation of growth in the strobilus and during its ripening, a vegetative lateral bud on the axis immediately below the strobilus is stimulated into activity, develops horizontal branches, and roots which grow down into the humus. When this new growth is thus made independent, the axis bearing the strobilus and the germinating embryos decays, and at the period when cotyledons and roots of the young sporophytes are thrust out of the sporangia, the strobilus is lying in contact with the ground, shaded and otherwise protected by the vigorous vegetative growth that is spread above them. The strobilus decays much more slowly than the leafy axis beneath. This accounts for my finding strobili which appear to be shed from the plants. In case a strobilus fails to produce embryos, either through failure in fertilization, or probably more often because the dry season overtakes the gametophyte at a critical stage of its growth, the strobili continue to grow indeterminately throughout the season, and apparently perform the vegetative work that, under more auspicious conditions, is taken up by the new growth from the lateral bud. Moreover, the spores rendered sterile by adverse conditions are shed from the sporangia. Thus not infrequently one finds greatly elongated strobili with a basal zone of empty gaping sporangia, surmounted by two or three whorls of empty microsporangia, then a zone of sprouting embryos, and an apical region of degenerating young stages of megasporangia. In contrast with this are the strobili which have developed under continuous favorable conditions, in which the *basal zone* displays embryos. Whether or not the strobilus may carry on its growth a third year in case of two successive failures to produce embryos, remains to be demonstrated. It will be a matter of interest to discover what variation in ecological conditions causes the strobili of the allied Texas species to pass the winter in a microsporangiate condition, whereas *S. rupestris* in Massachusetts appears to bear during the same season only megasporangia.

THE UNIVERSITY OF CHICAGO.

[*To be continued.*]